10/523,618

Machine Translation of DE 19919625:

Each year decease alone in the USA approx. 20,000 patients at the consequences of one Herzklappendysfunktion, and more than 60,000 patients are forced because of a Dysfunktion already recognized to let replace one or more Herzklappen operationally. When replacement for the own Herzklappe come either mechanical or biological flap prostheses (Xenografts) in question, become rarer kryopreservierte or glutaraldehydfixierte Homografts used. Mechanical flap prostheses however often lead to foreign body reactions with thromboembolischen complications, which are favoured by the flow conditions in the heart, changed with the artificial Herzklappe. Therefore a lifelong anti-coagulation of the patient concerned is necessary, who leads to a permanently increased bleeding danger. A further, often life-threatening complication with patients with a mechanical Herzklappe are infections.
A Herzklappe on the basis of a synthetic cell array structure is described in US 58 55 610. The matrix, which consists of a biocompatible, biologically degradable synthetic polymer and is so trained that it forms a mechanically loadable, flexible fabric structure, a receiver subkutan implantiert until an extracellular matrix formed. Subsequently, the matrix is removed again and is available then as Herzklappe.
With the Xenografts it mostly concerns pig flaps, which are treated with Glutaraldehyd. Curtil et al. (Journal OF Heart valve Disease 6, 296-306, 1997) describe the settlement of pig flaps with human fibroblasts and container endothelial cells, pre-treated by freezing drying process. WHERE 96/40889 A1 describes a procedure, with which lebensfähige cells can be introduced purposefully into a three-dimensional matrix from pig fabric, whereby the emphasis of the invention is on bringing in the cells into the matrix. Also for this procedure Herzklappen are mentioned as possible areas of application.
The production of a ?bioartifiziellen transplant?, which can be for example also a Herzklappe, is described in DE 198 28 726 A1. The manufacturing process plans compellingly, from allogenem or xenogenem fabric and/or. To possibly remove material antigens cells and settle afterwards either with cells of the intended transplant receiver or with genetically modified on the receiver cut cells. US 56 13 982 describes the production of bioprosthetischer Xenografts, which are suitable for the implantation in humans. As example of such Xenografts Herzklappen are indicated. In accordance with 56 13 982 procedures described in US the cells are removed from the xenogenen matrix and settled afterwards the matrix with allogenen or auto+lied cells, whereby the emphasis of the theory of this patent is with the treatment of the matrix with adhesion and growth factors.
Pig flap prostheses can be inserted with good results with older patients, are inclined however to degeneration after only approx. 12 to 15 years, so that they are not applicable for young people usually. Further a increased risk of infection exists with pig flap prostheses in the comparison to the healthy heart. Beyond that pig flaps are inclined to the Calcifizierung, why they are unsuitable to the employment at children and young people, who exhibit a increased calcium

metabolism. Finally they represent likewise body-strange fabric, which is recognized with a certain probability by the body-own immune system as strange and so that can release life-threatening immune processes.
As the third possibility Homografts, D stand. h. from human donors isolated, fixed Herzklappen, for the order. Homografts are resistant to infections relatively, represent however likewise body-strange fabric, which causes immune reactions with a certain probability. Beyond that Homografts are inclined just like pig flap prostheses to the Calcifizierung and are subject therefore to substantial degeneration which makes usually a Reoperation necessary after 7 to 12 years. Homografts are available beyond that to only extremely limited extent.
Apart from the disadvantages that so far as flap replacement used flap prostheses, D, already described. h. the release of immune reactions, the increased risk of infection, the danger of thromboembolischer processes and the Degenerationsneigung, it is common to all well-known flaps that they consist important characteristics of living matrix, z of inorganic material or fixed organic material and them therefore. B. the ability to Reparationsprozessen, for reconfiguration or to growth are missing. From this follows and. A. that with childlike flap patients so far regularly Reoperationen had to be taken in purchase. Additionally to each heart surgery the inherent risk rises however with each Reoperation the lethality risk, since by the preceding operations substantial growing together in the thorax arise.
There is therefore an urgent need for a heart flap replacement, which avoids the disadvantages described before. For this purpose was already suggested manufacturing artificial Herzklappen by ?tissue engineering?. The ?tissue engineering? is concerned with the development of ?biohybrid? implants, which grow up in the body to fabrics or to whole organ systems. First found the techniques ?tissue engineering? within the range of the Hauttransplantation application. In the meantime their application extended to other fabric, like liver, cartilage, bone and trachea as well as intestinale and urologische fabrics and blood vessels. Also to the production of biohybrid Herzklappen in the form of einelnen flap sails were already described; by ?tissue the heart folding gel manufactured it had engineering? however so far the disadvantage that they did not exhibit inadäquate, sufficient bindegewebige structures and therefore the flow conditions dominant in the heart after dissolution the biodegradablen carrier structure have withstood could not.
Task of the invention is it to place a procedure for manufacturing an artificial homologous Herzklappe for the order with which heart flap prostheses can be produced, which grew in vivo dominant flow conditions. A further task of the invention consists of making an improved Herzklappe available.
The first task is solved according to invention by in vitro procedures for manufacturing a homologous Herzklappe, which covers the following steps:

- Making a biologically degradable carrier available,
- Settling the carrier with homologous fibroblasts and/or Myofibroblasten for the training of a bindegewebigen matrix,

- Settling the carrier/the matrix with endothelial cells
- Bringing the carrier/the matrix into a pulsatile river chamber, whose river rate is changeable, and
- continuous or intermittent increase of the river rate.

Furthermore the invention makes a homologous Herzklappe available, which is producible by the procedure according to invention. Preferably the homologous Herzklappe covers a bindegewebigen internal core, which is surrounded by an endothelium cell layer, whereby in the bindegewebigen core a Kollagendichte is given from 43 to 55%, related to the matrix.
With the procedure according to invention homologous Herzklappen can be manufactured, which are suitable with the human patient due to a pronounced bindegewebigen core with naturenear kollagen, Elastin and Glycosaminanteil for the implantation. The procedure is to be described in the following more near.
In the following description the term ?carrier? means a azelluläre structure, which, as down is described more exactly, consists either of synthetic fibers or a azellulären Bindegewebsgerüst. The term ?matrix? designates a bindegewebige structure, which contains beside fibroblasts and Myofibroblasten typical components of an extracellular matrix, i.e. a Kollagen, a Elastin and a Glycosaminoglycane. With matrix designated structures contain typically no more girder elements. The transition stages between carriers and matrix are described by the double designation ?carrier/matrix?.
To the execution of the procedure according to invention first a biologically degradable carrier is made available. The substrate should be thereby on the one hand a certain time-long stably, over a sufficient settlement and/or. To make possible and the training of a bindegewebigen matrix to reach be able penetration with fibroblasts and/or Myofibroblasten, on the other hand within a justifiable time, which is anyhow smaller, than the time, which takes the education up of the homologous flap prosthesis, altogether hydrolytically can be dissolved. It is preferential that the hydrolytic dismantling after approximately. 8 days begins; it should errs the rule after 4 to 6 weeks to be final. With the substrate it concerns preferentially around a structure composed of polymer fibers, a porous polymer structure or a azelluläres biological fabric. Examples of biologically degradable substrates are Polyglycolsäure (PGA), Polymilchsäure (PLA), Polyhydroxyalkanoat (PHA) and Poly-4-Hydroxybutyrat (P4HB). These polymers can be used both purely and in mixtures from two or several of the substances mentioned or mixtures of these substances with further biologically degradable polymers. In a preferential execution form a mixing polymer from 85% PGA and 15% PLA is used. Ideal way is forwards formed the biologically degradable carrier in the form of the desired Herzklappe. Examples of possible heart flap forms are in fig. 1 indicated.
It proved as meaningful, carriers with a polymer density of approx. 40 to 120 mg/cm< 3> to use. Below 40 mg/cm< 3> the polymer fabric is too unstably, over-half mg/cm< of 120; 3> the fabric is too close, in order to permit the penetration of fibroblasts within a justifiable period. In preferential execution forms the density of the biologically degradable carrier 50 to 80 amounts to mg/cm< 3> ,

particularly prefers 70 mg/cm< 3>. From the inventors a polymere carrier of the companies became with good results Albany internationally Research, Mensville, mA, the USA, with a density of approx. 70 mg/cm< 3> used. The fibers of the carrier can have a diameter from 6 to 20 mu m, prefer 10 to 18 mu M. There is however also fabrics with other fiber strengths conceivablly, which must lend however on the one hand a certain stability to the carrier, on the other hand settling and penetration of the carrier with fibroblasts or Myofibroblasten to permit must. Further porous (spongelike) polymer forms can be used. Here Porengrössen proved preferentially of 80-240 mu m as. The pores can be obtained by the so-called ?salt Leaching? technology, which admits to the specialist is.

Instead of a synthetic carrier, as described before, the use of a azellulären Bindegewebsgerüstes is conceivable. So for example a pig flap could be converted into an immunologically neutral fabric (baths et al., Eur. J. - Cardiothorac. Surg. 14, 279, 1998), which could be settled afterwards with homologous cells.

The biologically degradable carrier is inkubiert first with a fibroblast population. In the case of use of homologous fibroblasts and/or Myofibroblasten, D. h. by fibroblasts and/or Myofibroblasten from humans, but not necessarily the patient, should be paid attention to same HLA classification. Fibroblast populations know thereby z. B. from peripheral blood vessels, both Arterien and Venen, to be won. For this in particular the Arteria offers itself radialis the lower arm, which in most cases stands because of the arterial double supply of the arm to the Explantation without loss for order. Alternatively container cells from blood vessels of the leg, z can. B. the Vena saphena, to be won. Is conceivable the far production cells manipulated genetically by fibroblasts or Myofibroblasten from pluripotenten main cells or.

The cells can be won for example from container fragments, in that the fabric bits first, as in ignite et al. (Eur J. Cardiothorac Surg. 13, 160, 1998) described, into pieces of fabric break carves up and approx. 1 to 2 weeks on normal cell culture conditions (37 DEG C, 5CO2, 95% air humidity) to be inkubiert, until the cells on the soil of the culture dish form a konfluente cell layer. Subsequently, they are subjected repeated passages, in order to receive a cell culture free of remaining fabric material. According to two to three passages the mixed cell populations can be cleaned, be inkubiert in that them with for endothelial cells specific fluorescence marker (Dil AC LDL, of Medical Technologies Inc., Stoughton, mA) and be separated by means of Durchflusszytometrie (FACStar plus, Becton Dickinson). Fluorescence-marked cells are endothelial cells, not marked cells are fibroblasts and Myofibroblasten. These are cultivated further two to three weeks and during this time two to four passages subjected, in order to receive a sufficient number of cells for following settling of the carrier.

In in such a way received fibroblast cultures fibroblasts and Myofibroblasten are present mixed. The relationship of both populations varies. It is preferential using cultures in which the fibroblast portion outweighs; a fibroblast portion of more than 75% is particularly preferential. A as described cleaned or every other pure fibroblasts/Myofibroblastenkultur can be used now for settling the polymer carrier.

In addition become per square centimeter of the carrier approx. 105 to 5 x 10< 8> Fibroblasts and/or Myofibroblasten assigned. Under ?surface? the surface recognizable with view of the carrier from above in one level is meant in this case not the actual surface of the polymer, but. Usually become the fibroblasts 60 to 90 min. Time given, around itself to the carrier anzuheften. Subsequently, the supernatant medium can be removed and be admitted a further mark fibroblast suspension. Ideal way one leaves however between the first and second addition of fibroblast suspension 2 to 36 hours, prefers 24 hours applying.
In a preferential execution form of the procedure according to invention become the carrier and/or. after the first fibroblast addition gradually training the matrix further 3 to 14 times, particularly 5 to 10 times fibroblasts and/or Myofibroblasten prefers themselves caused.
Under the conditions usually used for the cell growth of fibroblasts (z. B. 5% CO2, Inkubation with 37 DEG C, sterile medium) develops after approximately. until three weeks a solid bindegewebige structure. Now this structure with a pure endothelium cell suspension is inkubiert. The endothelial cells can being enriched exactly the same as fibroblasts by FACS and be expanded afterwards in several passages (prefers 3). Also for endothelial cells it is preferential, settling with approx. in each case. 10< 5> to 5 x 10< 8> To repeat endothelial cells several times, z. B. 3 to 14 times. In preferential execution forms settling with endothelial cells 5 to is 10 times repeated. Between two settling steps at least 60 min. should be appropriate, preferentially however for 2 to 24 hours.
With the cells used for settling the carrier it concerns preferentially human cells. Particularly preferentially it is however to use autologe fibroblasts and/or Myofibroblasten as well as endothelial cells. In addition the patient, whose Herzklappe is to be replaced, fabric becomes z. B. taken out of one of its containers. As already mentions above, for it the Arteria offers itself radialis as well as the Vena saphena. The use of the auto+lied cells for the construction of the Herzklappe has the substantial advantage that the flap represents after implantation in the patients no body-strange fabric and thus immune reactions against the artificial Herzklappe appears so well impossible.
Approx. Histologically and immune-histochemically a heart-fold-similar fabric with superfiziellen single cell layer from endothelial cells and a bindegewebigen essential structure can 14 days after addition of the endothelial cells be proven. This fabric is however suitable for the implantation into a human heart only conditionally, since it would not have been up to to the there dominant flow conditions due to its to weak mechanical characteristics.
Now in a further process step the trained heart-fold-similar structure is brought in according to invention into a pulsatile river chamber, in which it can be suspended rising river rates. It was stated that by a slow adaptation of the river rates the education of a flowsteady heart-fold-similar structure can be achieved. For accomplishing the procedure according to invention a bioreactor is suitable. This bioreactor should be as compactly as possible trained, in order to be able to be used in usual Zellinkubatoren. The bioreactor should exhibit a river chamber, in which the flap valve is arranged. The drive for pumping the fluid by the river chamber should be arranged thereby outside of the river chamber. This is

obtained with the used bioreactor by one the river chamber neighbouring Luftkammer, which is separate from the river chamber by a high elastic diaphragm. By pulsating changing of the air pressure in the Luftkammer promoting the fluid can be produced by the river chamber. In place of air also liquid or another gas could be taken up in the Luftkammer, which can be called then generally also means of driving chamber. Then this gas or the fluid pressure changes pulsating in it in each case would have to be subjected. Air can be handled particularly easy however and therefore as particularly suitably for the operation of the bioreactor proved.
Due to the Luftkammer the drive can be arranged very compact for promoting the fluid in the river chamber and be arranged also outside of the Inkubators. Thereby a separation can be caused between the part, which affects the river chamber directly, and which part, that the pressure fluctuations in the Luftkammer, and/or. Means of driving chamber produces. So z can. B. a Respiratorpumpe outside of the Inkubators arranged its, those over a thin hose with the Luftkammer, and/or. Means of driving chamber is connected. Thus the organization of the bioreactor and the drive can be adapted to the respective installation requirements.
According to invention the pulsatile river chamber used for the procedure is a component of a bioreactor. A preferential execution form of the bioreactor becomes following with reference to fig. 2 and 3 described in detail. It understands itself that both the bioreactor and it the comprehensive arrangement could be used not only in the procedure according to invention, but also independently of it.
Show:
Fig. 1a a carrier trained from a polymer, which is subjected afterwards to a settlement with fibroblasts/Myofibroblasten and endothelial cells;
Fig. 1b the carrier/the matrix after settling and one week Inkubation in the pulsatilen river chamber;
Fig. 1K the same flap prosthesis after 2 weeks Inkubation in the river chamber.
Fig. 2 the bioreactor including a pulsatilen river chamber in a cutaway view;
Fig. 3 an arrangement for the operation of the bioreactor from fig. 2.
In fig. a bioreactor 1 is represented 2 in a cutaway view. The bioreactor is around in fig. 2 represented vertical symmetry axis essentially rotationally symmetrically. It concerns with the bioreactor a so-called compact bioreactor, its outside diameters in radial direction approx. 15.5 cm and its height approx. 16.8 cm amounts to. The enterprise can take place also in standard Zellinkubatoren.
The bioreactor 1 orders 2, with two chambers 3 and 4, which are by diaphragm 5 from each other separated over a housing. The lower chamber 3 forms a Luftkammer and the upper chamber 4 a river chamber, which is divided into two parts, whereby a part is formed by a fluid chamber section 6 and the other part by a valve perfusion chamber section 7. The fluid chamber section 6 and the valve perfusion chamber section 7 are connected by a passage 8.
The chambers communicate with the environment over connections 9, 10 and 11, whereby connection 9 into the chamber 3, connection 10 flows into the fluid chamber section 6 of the river chamber 4 and connection 11 into the valve

perfusion chamber section 7 of the river chamber 4. The connections 9, 10 and 11 are designed as tube ends managing over the housing in each case, on which hoses or lines are plug-on in well-known way.
The housing is three-part trained, with a lower Gehäuseteil 12, a middle Gehäuseteil 13 and an upper Gehäuseteil 14. Lower Gehäuseteil 12 is essentially dish-shaped with a soil 15 and an essentially circularly running wall 16. Those the wall 16 turned away side of the soil forms at the same time the bearing surface for the bioreactor, with which he z. B. on a table to be touched down can. The connection 9 extends essentially radially from the wall 16 out. The wall 16 exhibits a flange surface 17, in which in axial direction extending tapped holes 18 are let in. In order to facilitate an observing of the procedures inside the chamber 3, is lower Gehäuseteil from transparent material, z. B. Plexiglass (Polymethylmetacrylat, PMMA), manufactured.
Middle Gehäuseteil 13 is likewise essentially rotationally symmetrically had turned away side of the wall 19 around the symmetry axis of the bioreactor and over an essentially cylindrical wall 19, which a cover section 20 follows, as well as a flange 21 on that the cover section 20. The flange 21 is essentially circular with one the flange surface 17 turned flange surface 22, which extends radially. In the flange 21 are besides through-holes 23 intended, aligning one of the tapped holes 18 is assigned to which in each case. The through-holes 23 and tapped holes 18 are evenly distributed at the extent, whereby the preferential execution form has 18 with associated through-holes 23 nine tapped holes.
Between the two flange surfaces 17 and 22 is the diaphragm 5. By the diaphragm 5 the two chambers 3 and 4 are separated. The diaphragm consists of high elastic silicone, which is strained in the kind of a drum skin between the flange surfaces 17 and 22. The diaphragm points a thickness from approx. 0.5 mm up, and it can be so trained with the fact that in the installed condition its outside diameter essentially corresponds to the outside diameter of the lower and the middle Gehäuseteils. Then the diaphragm must be provided with openings in the range of the through-holes 23. In order to clamp the diaphragm 5 firmly between the flange surfaces 17 and 22, by the through-holes 23 screws 24 from stainless steel into the tapped holes 18 are screwed in, which extend also by the openings through diaphragm. By the screws 24 the flange surfaces 22 and 17 are pressed against each other, in order to clamp thereby the diaphragm firm and to obtain optimal tightness.
During the enterprise of the bioreactor the diaphragm in axial direction swings. In order to reduce the load of the diaphragm at the transition of the flange surface 17 and 22 to the assigned walls 16 and 19, circulating there in each case phases 25 and 26 are intended.
The fluid chamber section 6 is limited upward thus downward by the diaphragm 5 and by the cover section 20. In order to be able to supervise the reactions in the fluid chamber section 6 visually, middle Gehäuseteil, like also lower Gehäuseteil of transparent material the z consists. B. Plexiglass.
Middle Gehäuseteil 13 follows upper Gehäuseteil 14. This is essentially bell-shaped and by a flange connection with the middle Gehäuseteil connected. For this flange connection middle Gehäuseteil has 13 in its cover section six tapped

holes 27, which extend in axial direction and are evenly distributed at the extent. The tapped holes 27 are arranged thereby in a radially running flange surface 28, which exhibits a circulating groove 29 for the admission of a sealing element. Such a sealing element knows z. B. an O-ring its. The groove 29 is radially inward transferred in relation to the tapped holes 27.
Upper Gehäuseteil 14 has turned flange surface 32 a flange ring 30 with through-holes 31 and one the flange surface 28. Upper Gehäuseteil 14 rises with its flange surface 32 on the flange surface 28 and is by screws 33 from stainless steel, which are put by the through-holes 31 and screwed in in each case into the tapped holes 27, with the middle Gehäuseteil 13 firmly connected. The sealing element is pressed thereby by the flange surface 32 into the groove 29 and seals the valve perfusion chamber section 7 to the environment. The connection 11 is arranged in the point of the upper Gehäuseteils 14. Similar to the two other Gehäuseteile 12 and 13 upper Gehäuseteil consists 14 of transparent material z. B. Plexiglass.
As mentions already initially, the fluid chamber section 6 and the valve perfusion chamber section 7 connected by a passage 8 are. This passage 8 is formed by a through-hole 34 in the cover section 20 of the middle Gehäuseteils 13. Into this through-hole 34, which extends axially, a pipe 35 is pushed in, that itself extended over the cover section 20 outside. On the tubing section of the pipe 35, which extends over the cover section 20 of the middle Gehäuseteils 13 outside, a silicone pipe 36 is attached. This silicone pipe is removable. At the outside diameter of the silicone pipe a circular stage 37 is intended. By the organization of the silicone pipe 36 and the stage 37 it is possible to attach valves to the silicone pipe 36. Also it is possible to put into the silicone pipe 36 valves or filters since the pipe 35 exhibits a smaller inside diameter than the silicone pipe 36 or thus a contact surface for a filter or such a thing the face of the pipe 35 can form. In the available case the valve is a 2-segeliges or a 3-segeliges flap valve, that not represented more near the carrier described above, and/or. corresponds to the matrix, and as check valve works, in order to make possible a fluid stream only from the fluid chamber section to the valve perfusion chamber section. The Klappenkonstrukt of the flap valve is inserted on a silicone ring. For fastening the flap valve to the silicone pipe the silicone ring is put on the silicone pipe and held by frictional engagement.
At the lower surface of the cover section a circular recess 38 is intended, which runs concentrically to the pipe 35 and flows into those the pipe 35. This recess 38 knows z. B. as admission for filter materials or such a thing serve.
In the further a bioreactor arrangement with the bioreactor 1 described above is described. For the operation of the bioreactor a Respiratorpumpe 39 is intended, which is connected by a silicone hose 40 and the connection 9 with the chamber 3. The Respiratorpumpe produces adjustable pressure impulses, by which the pressure of the chamber can be periodically increased. The Respiratorpumpe is steered two-phases a Respiratorpumpe, which functions as tire pump. With the pump the pumping volume and the pumping frequency can be stopped, whereby the river lies in a range from 50 ml per minute to 5000 ml per minute, and the system pressure between 20 and 240 mmHg to vary can.

A reservoir 41 is connected by silicone hoses 42 and 43 with the connections 10 and 11 in each case. From the silicone hoses 42 and 43 and the reservoir 41 a cycle results, whereby fluid from the valve perfusion chamber section 7 by means of the connection 10, the silicone hose 42 to the reservoir 41 and from there out by means of the silicone hose 43 can be promoted and the connection 10 to the fluid chamber section 6.
The bioreactor 1 and the reservoir 41 with the silicone hoses 42 and 43 are in a standardized Inkubator 44 with 37 DEG C and 5% CO2.
In the following the effect and function mode of the bioreactor are more near described:
Over the Respiratorpumpe 39 pressure impulses are led into the chamber 3. Due to these pressure impulses the diaphragm expands 5, whereby it itself in the representation in fig. 2 with each pressure pressure upward curves and at it also to an increase of pressure in the fluid chamber section 6 of the river chamber 4 leads. This increase of pressure is passed on into the passage 8 and does not open thereby the flap valve at the silicone pipe 36, represented not more near. In this way the fluid existing in the fluid chamber section 6 is promoted to the valve perfusion chamber section 7. From the valve perfusion chamber section 7 fluid is then promoted by means of the connection 11 and the silicone hose 42 to the reservoir 11. From there out it can be returned by means of the silicone hose 43 to the fluid chamber section 6. With sloping pressure in the chamber 3 the diaphragm 5 is backtransferred again due to its internal voltage into their starting position, into which it essentially radially extends. Via the decrease of pressure a decrease of pressure also in the fluid chamber section 6, which leads again to a reasoning as flap valve of trained valve, takes place.
Thus a cycle can be produced by the pulsating increase of pressure in the chamber, essentially the physiological river conditions in the heart simulated.
Due to the construction of the new bioreactor a high tightness of the fluid chamber section 6 and the valve perfusion chamber section 7 is reached. Due to the protection from infections, possible thereby, long cultivation times can be made possible. By the drive with air over the chamber 3, which is hermetically from the fluid chamber 6 separate by the diaphragm 5, the problems of heat developments can be avoided within the Inkubators 44, there the actual pumping engine (Respiratorpumpe) outside of the Zellinkubators are.
Since the entire bioreactor is transparency, one can see the flap construction permanently and control an opening and a closing of the flaps. Further one can recognize pH changes in the colouring of the nutrient fluid.
The reservoir with the fluid can be attached by sterile connectors to the silicone hoses. Thus a safe fluid change can be made possible.
Due to the simple construction of the bioreactor an exchange of the valves can and/or. the Herzklappen to be accomplished in a simple manner. In addition only the screws 33 must be solved and upper Gehäuseteil 14 be removed. The valve and/or. the Herzklappe can be exchanged then and afterwards can upper Gehäuseteil 14 by the screws 33 again to the middle Gehäuseteil be fastened.
In an execution form of the invention river rates between 50 become ml/min. and 5000 ml/min., prefers 50 ml/min. until 2000 ml/min use. The data refer to the river

by the flap prosthesis. As initial river rate river rates from 50 to 100 have themselves ml/min. as suitably proved. These river rates become z. B. with a pulse frequency by 5 to 10 pulses per minute by the Herzklappe skillfully. The river rate becomes afterwards continuously or intermittent on up to 5000 ml/min. increased. The pulse frequency on up to 180 pulses/min. becomes simultaneous. raised. With the indicated data it concerns the limit values, which are not crossed normally.

In preferential execution forms the river rate becomes up to 2000 ml/min. increased, while the pulse frequency on 70 to 100 prefers, 80 pulses/min. one raises. The load of the stabilizing Herzklappe is adapted thereby to almost physiological conditions. It has itself as favorable, but, the river rate and the pulse frequency not necessarily proved in each case after approximately. to increase 24 to 48 hours. So can for example, on the basis of a river rate from 50 to 100 ml/min. and a pulse rate of 5 to 10 pulses/min. on the day 1 of the stay in the pulsatilen river chamber, on the day 3 an increase on 300 ml/min. with 20 to 25 pulses/min., on the day 5 to 700 ml/min. and 35 to 45 pulses/min., on the day 7 on 1000 ml/min and 50 to 60 pulses/min., on the day 9 on 1300 ml/min. and 70 to 80 pulses/min., on the day 11 on 1500 ml/min. and approx. 100 pulses/min., on the day 13 on 1750 ml/min. and approx. 120 pulses/min. and on the day 15 on 2000 ml/min. and 140 pulses/min. are planned. Depending upon for the order of standing time, size of the flap, size and the age of the patient etc. however a very much slower increase of the river rates as well as the pulse frequency can be meaningful or the increase on higher river rates and pulse frequencies.

In an execution form of the invention the systemic pressures dominant in the pulsatilen river chamber are stopped to 10 to 240 mmHg. Systemic pressures are preferential preferentially from 60 to 140 particularly are systemic pressures from 80 to 120 mmHg.

According to invention the homologous manufactured by means of the procedure and/or. autologe Herzklappe exhibits substantial advantages opposite the conventional mechanical and biological Herzklappen. Thus it consists in its preferential execution form of auto+lied fabrics, D. h. made of fabric of the patient waiting for the heart flap operation, and thereby each foreign body reaction of the flap receiver avoids. The risk of infection with receivers of autologer Herzklappen differs not from the one healthy heart. A Antikoagolationstherapie is not necessary; thus the danger of hemorrhagischer complications is void. The by far most convincing advantage of the Herzklappe according to invention is however the fact that it represents living fabric and therefore after implantation with growth of the heart flap receiver along. That makes the Herzklappe according to invention the flap of the choice particularly with children and juvenile patients, whose heart development still becomes larger. The living Herzklappe grows accordingly also, so that also on changes of the heart no Dysproportionen (z. B. Arise to narrowings) between flap and heart.

The Herzklappe according to invention contains a bindegewebige internal structure, which essentially contains components of a normal extracellular matrix, i.e. Kollagen, a Elastin and a Glycosaminoglykane beside fibroblasts and Myofibroblasten. Contrary to earlier attempts to manufacture by ?tissue

engineering? heart flap fabric the flaps according to invention point to one the native flap and/or. the native folding gel appropriate portion of Kollagen (43-55%), Elastin (11-13%) and Glycosaminoglycan up.
It could be shown that the Herzklappen according to invention river rates of more than 2000 ml/min., according to river conditions dominant in an adult human heart, to withstand. Thus a autologe Herzklappe can be made available for the first time according to invention, which is unconditionally for the implantation into childlike like also adults patients suitable.
The following examples describe the invention.
Example 1

Production of carriers

Manufacturing the herzklappenförmigen carrier a non-woven Polyglycolsäurepolymer (fiber diameter becomes: 12-15 mu m, polymer density: 70 mg/ml, Albany internationally Research, Mansfield mA, the USA.) uses. The polymer is cut in the kind that it forms a tube with 19 mm in diameter. In this Conduit 3 triangular sails are inserted. This carrier can for manufacturing 3-segeligen flaps, D. h. Pulmonal, Aorten and Tricuspidalklappen to be used. For Mitralklappen 2 sails are inserted.
Example 2

Production of a dreisegeligen heart flap prosthesis

A dreisegeliger carrier is sterilized and inserted into medium (DMEM, GIBCO BRL running Technologies) for 24 hours, in order to in-soft the polymer surface. Thereupon the klappenförmige carrier per square centimeter becomes surface with 4 millions Fibroblasts every 90 minutes altogether 6 times settles. Further the settled carrier is inkubiert for 2 weeks (5% CO2, 37 DEG C, 95% air humidity). The medium is changed every 4 days under sterile conditions. Subsequently, endothelial cells are applied on the settled klappenförmigen carrier (3-4 millions Endothelial cells per square centimeter surface, 6 settlements every 90 minutes). After further 2 weeks the developed fabric is brought into the river chamber the bioreactor under sterile safeguard clauses and installed here by means of the silicone ring into flow position. Subsequently, the bioreactor is filled with medium and placed into the Zellinkubator. After over the compressed air hose the Konnektion was manufactured to the pump standing outside of the Inkubators, with minimum pulsatilen rivers one begins (50 ml/min). In 2 daily steps the river rate and pulse rate increased on 100 ml/min (pulse 10), 300 ml (pulse 25), 700 ml (pulse 35), 1000 ml (pulse 60) for altogether far 4 days. Subsequently, (after 14 days) the fabric under sterile conditions, formed now, is taken and asserviert to the biochemical, histological and mechanical analysis.

Each year decease alone in the USA approx. 20,000 patients at the consequences of one Herzklappendysfunktion, and more than 60,000 patients are forced because of a Dysfunktion already recognized to let replace one or more Herzklappen operationally. When replacement for the own Herzklappe come either mechanical or biological flap prostheses (Xenografts) in question, become rarer kryopreservierte or glutaraldehydfixierte Homografts used. Mechanical flap prostheses however often lead to foreign body reactions with thromboembolischen complications, which are favoured by the flow conditions in the heart, changed with the artificial Herzklappe. Therefore a lifelong anti-coagulation of the patient concerned is necessary, who leads to a permanently increased bleeding danger. A further, often life-threatening complication with patients with a mechanical Herzklappe are infections.
A Herzklappe on the basis of a synthetic cell array structure is described in US 58 55 610. The matrix, which consists of a biocompatible, biologically degradable synthetic polymer and is so trained that it forms a mechanically loadable, flexible fabric structure, a receiver subkutan implantiert until an extracellular matrix formed. Subsequently, the matrix is removed again and is available then as Herzklappe.
With the Xenografts it mostly concerns pig flaps, which are treated with Glutaraldehyd. Curtil et al. (Journal OF Heart valve Disease 6, 296-306, 1997) describe the settlement of pig flaps with human fibroblasts and container endothelial cells, pre-treated by freezing drying process. WHERE 96/40889 A1 describes a procedure, with which lebensfähige cells can be introduced purposefully into a three-dimensional matrix from pig fabric, whereby the emphasis of the invention is on bringing in the cells into the matrix. Also for this procedure Herzklappen are mentioned as possible areas of application.
The production of a ?bioartifiziellen transplant?, which can be for example also a Herzklappe, is described in DE 198 28 726 A1. The manufacturing process plans compellingly, from allogenem or xenogenem fabric and/or. To possibly remove material antigens cells and settle afterwards either with cells of the intended transplant receiver or with genetically modified on the receiver cut cells. US 56 13 982 describes the production of bioprosthetischer Xenografts, which are suitable for the implantation in humans. As example of such Xenografts Herzklappen are indicated. In accordance with 56 13 982 procedures described in US the cells are removed from the xenogenen matrix and settled afterwards the matrix with allogenen or auto+lied cells, whereby the emphasis of the theory of this patent is with the treatment of the matrix with adhesion and growth factors. Pig flap prostheses can be inserted with good results with older patients, are inclined however to degeneration after only approx. 12 to 15 years, so that they are not applicable for young people usually. Further a increased risk of infection exists with pig flap prostheses in the comparison to the healthy heart. Beyond that pig flaps are inclined to the Calcifizierung, why they are unsuitable to the employment at children and young people, who exhibit a increased calcium metabolism. Finally they represent likewise body-strange fabric, which is recognized with a certain probability by the body-own immune system as strange and so that can release life-threatening immune processes.

As the third possibility Homografts, D stand. h. from human donors isolated, fixed Herzklappen, for the order. Homografts are resistant to infections relatively, represent however likewise body-strange fabric, which causes immune reactions with a certain probability. Beyond that Homografts are inclined just like pig flap prostheses to the Calcifizierung and are subject therefore to substantial degeneration which makes usually a Reoperation necessary after 7 to 12 years. Homografts are available beyond that to only extremely limited extent.
Apart from the disadvantages that so far as flap replacement used flap prostheses, D, already described. h. the release of immune reactions, the increased risk of infection, the danger of thromboembolischer processes and the Degenerationsneigung, it is common to all well-known flaps that they consist important characteristics of living matrix, z of inorganic material or fixed organic material and them therefore. B. the ability to Reparationsprozessen, for reconfiguration or to growth are missing. From this follows and. A. that with childlike flap patients so far regularly Reoperationen had to be taken in purchase. Additionally to each heart surgery the inherent risk rises however with each Reoperation the lethality risk, since by the preceding operations substantial growing together in the thorax arise.
There is therefore an urgent need for a heart flap replacement, which avoids the disadvantages described before. For this purpose was already suggested manufacturing artificial Herzklappen by ?tissue engineering?. The ?tissue engineering? is concerned with the development of ?biohybrid? implants, which grow up in the body to fabrics or to whole organ systems. First found the techniques ?tissue engineering? within the range of the Hauttransplantation application. In the meantime their application extended to other fabric, like liver, cartilage, bone and trachea as well as intestinale and urologische fabrics and blood vessels. Also to the production of biohybrid Herzklappen in the form of einelnen flap sails were already described; by ?tissue the heart folding gel manufactured it had engineering? however so far the disadvantage that they did not exhibit inadäquate, sufficient bindegewebige structures and therefore the flow conditions dominant in the heart after dissolution the biodegradablen carrier structure have withstood could not.
Task of the invention is it to place a procedure for manufacturing an artificial homologous Herzklappe for the order with which heart flap prostheses can be produced, which grew in vivo dominant flow conditions. A further task of the invention consists of making an improved Herzklappe available.
The first task is solved according to invention by in vitro procedures for manufacturing a homologous Herzklappe, which covers the following steps:

- Making a biologically degradable carrier available,
- Settling the carrier with homologous fibroblasts and/or Myofibroblasten for the training of a bindegewebigen matrix,
- Settling the carrier/the matrix with endothelial cells
- Bringing the carrier/the matrix into a pulsatile river chamber, whose river rate is changeable, and

- continuous or intermittent increase of the river rate.

Furthermore the invention makes a homologous Herzklappe available, which is producible by the procedure according to invention. Preferably the homologous Herzklappe covers a bindegewebigen internal core, which is surrounded by an endothelium cell layer, whereby in the bindegewebigen core a Kollagendichte is given from 43 to 55%, related to the matrix.
With the procedure according to invention homologous Herzklappen can be manufactured, which are suitable with the human patient due to a pronounced bindegewebigen core with naturenear kollagen, Elastin and Glycosaminanteil for the implantation. The procedure is to be described in the following more near.
In the following description the term ?carrier? means a azelluläre structure, which, as down is described more exactly, consists either of synthetic fibers or a azellulären Bindegewebsgerüst. The term ?matrix? designates a bindegewebige structure, which contains beside fibroblasts and Myofibroblasten typical components of an extracellular matrix, i.e. a Kollagen, a Elastin and a Glycosaminoglycane. With matrix designated structures contain typically no more girder elements. The transition stages between carriers and matrix are described by the double designation ?carrier/matrix?.
To the execution of the procedure according to invention first a biologically degradable carrier is made available. The substrate should be thereby on the one hand a certain time-long stably, over a sufficient settlement and/or. To make possible and the training of a bindegewebigen matrix to reach be able penetration with fibroblasts and/or Myofibroblasten, on the other hand within a justifiable time, which is anyhow smaller, than the time, which takes the education up of the homologous flap prosthesis, altogether hydrolytically can be dissolved. It is preferential that the hydrolytic dismantling after approximately. 8 days begins; it should errs the rule after 4 to 6 weeks to be final. With the substrate it concerns preferentially around a structure composed of polymer fibers, a porous polymer structure or a azelluläres biological fabric. Examples of biologically degradable substrates are Polyglycolsäure (PGA), Polymilchsäure (PLA), Polyhydroxyalkanoat (PHA) and Poly-4-Hydroxybutyrat (P4HB). These polymers can be used both purely and in mixtures from two or several of the substances mentioned or mixtures of these substances with further biologically degradable polymers. In a preferential execution form a mixing polymer from 85% PGA and 15% PLA is used. Ideal way is forwards formed the biologically degradable carrier in the form of the desired Herzklappe. Examples of possible heart flap forms are in fig. 1 indicated.
It proved as meaningful, carriers with a polymer density of approx. 40 to 120 mg/cm< 3> to use. Below 40 mg/cm< 3> the polymer fabric is too unstably, over-half mg/cm< of 120; 3> the fabric is too close, in order to permit the penetration of fibroblasts within a justifiable period. In preferential execution forms the density of the biologically degradable carrier 50 to 80 amounts to mg/cm< 3> , particularly prefers 70 mg/cm< 3>. From the inventors a polymere carrier of the companies became with good results Albany internationally Research, Mensville, mA, the USA, with a density of approx. 70 mg/cm< 3> used. The fibers of the

carrier can have a diameter from 6 to 20 mu m, prefer 10 to 18 mu M. There is however also fabrics with other fiber strengths conceivablly, which must lend however on the one hand a certain stability to the carrier, on the other hand settling and penetration of the carrier with fibroblasts or Myofibroblasten to permit must. Further porous (spongelike) polymer forms can be used. Here Porengrössen proved preferentially of 80-240 mu m as. The pores can be obtained by the so-called ?salt Leaching? technology, which admits to the specialist is.

Instead of a synthetic carrier, as described before, the use of a azellulären Bindegewebsgerüstes is conceivable. So for example a pig flap could be converted into an immunologically neutral fabric (baths et al., Eur. J. - Cardiothorac. Surg. 14, 279, 1998), which could be settled afterwards with homologous cells.

The biologically degradable carrier is inkubiert first with a fibroblast population. In the case of use of homologous fibroblasts and/or Myofibroblasten, D. h. by fibroblasts and/or Myofibroblasten from humans, but not necessarily the patient, should be paid attention to same HLA classification. Fibroblast populations know thereby z. B. from peripheral blood vessels, both Arterien and Venen, to be won. For this in particular the Arteria offers itself radialis the lower arm, which in most cases stands because of the arterial double supply of the arm to the Explantation without loss for order. Alternatively container cells from blood vessels of the leg, z can. B. the Vena saphena, to be won. Is conceivable the far production cells manipulated genetically by fibroblasts or Myofibroblasten from pluripotenten main cells or.

The cells can be won for example from container fragments, in that the fabric bits first, as in ignite et al. (Eur J. Cardiothorac Surg. 13, 160, 1998) described, into pieces of fabric break carves up and approx. 1 to 2 weeks on normal cell culture conditions (37 DEG C, 5CO2, 95% air humidity) to be inkubiert, until the cells on the soil of the culture dish form a konfluente cell layer. Subsequently, they are subjected repeated passages, in order to receive a cell culture free of remaining fabric material. According to two to three passages the mixed cell populations can be cleaned, be inkubiert in that them with for endothelial cells specific fluorescence marker (Dil AC LDL, of Medical Technologies Inc., Stoughton, mA) and be separated by means of Durchflusszytometrie (FACStar plus, Becton Dickinson). Fluorescence-marked cells are endothelial cells, not marked cells are fibroblasts and Myofibroblasten. These are cultivated further two to three weeks and during this time two to four passages subjected, in order to receive a sufficient number of cells for following settling of the carrier.

In in such a way received fibroblast cultures fibroblasts and Myofibroblasten are present mixed. The relationship of both populations varies. It is preferential using cultures in which the fibroblast portion outweighs; a fibroblast portion of more than 75% is particularly preferential. A as described cleaned or every other pure fibroblasts/Myofibroblastenkultur can be used now for settling the polymer carrier. In addition become per square centimeter of the carrier approx. 105 to 5 x 10< 8> Fibroblasts and/or Myofibroblasten assigned. Under ?surface? the surface recognizable with view of the carrier from above in one level is meant in this case

not the actual surface of the polymer, but. Usually become the fibroblasts 60 to 90 min. Time given, around itself to the carrier anzuheften. Subsequently, the supernatant medium can be removed and be admitted a further mark fibroblast suspension. Ideal way one leaves however between the first and second addition of fibroblast suspension 2 to 36 hours, prefers 24 hours applying.
In a preferential execution form of the procedure according to invention become the carrier and/or. after the first fibroblast addition gradually training the matrix further 3 to 14 times, particularly 5 to 10 times fibroblasts and/or Myofibroblasten prefers themselves caused.
Under the conditions usually used for the cell growth of fibroblasts (z. B. 5% CO2, Inkubation with 37 DEG C, sterile medium) develops after approximately. until three weeks a solid bindegewebige structure. Now this structure with a pure endothelium cell suspension is inkubiert. The endothelial cells can being enriched exactly the same as fibroblasts by FACS and be expanded afterwards in several passages (prefers 3). Also for endothelial cells it is preferential, settling with approx. in each case. 10< 5> to 5 x 10< 8> To repeat endothelial cells several times, z. B. 3 to 14 times. In preferential execution forms settling with endothelial cells 5 to is 10 times repeated. Between two settling steps at least 60 min. should be appropriate, preferentially however for 2 to 24 hours.
With the cells used for settling the carrier it concerns preferentially human cells. Particularly preferentially it is however to use autologe fibroblasts and/or Myofibroblasten as well as endothelial cells. In addition the patient, whose Herzklappe is to be replaced, fabric becomes z. B. taken out of one of its containers. As already mentions above, for it the Arteria offers itself radialis as well as the Vena saphena. The use of the auto+lied cells for the construction of the Herzklappe has the substantial advantage that the flap represents after implantation in the patients no body-strange fabric and thus immune reactions against the artificial Herzklappe appears so well impossible.
Approx. Histologically and immune-histochemically a heart-fold-similar fabric with superfiziellen single cell layer from endothelial cells and a bindegewebigen essential structure can 14 days after addition of the endothelial cells be proven. This fabric is however suitable for the implantation into a human heart only conditionally, since it would not have been up to to the there dominant flow conditions due to its to weak mechanical characteristics.
Now in a further process step the trained heart-fold-similar structure is brought in according to invention into a pulsatile river chamber, in which it can be suspended rising river rates. It was stated that by a slow adaptation of the river rates the education of a flowsteady heart-fold-similar structure can be achieved. For accomplishing the procedure according to invention a bioreactor is suitable. This bioreactor should be as compactly as possible trained, in order to be able to be used in usual Zellinkubatoren. The bioreactor should exhibit a river chamber, in which the flap valve is arranged. The drive for pumping the fluid by the river chamber should be arranged thereby outside of the river chamber. This is obtained with the used bioreactor by one the river chamber neighbouring Luftkammer, which is separate from the river chamber by a high elastic diaphragm. By pulsating changing of the air pressure in the Luftkammer

promoting the fluid can be produced by the river chamber. In place of air also liquid or another gas could be taken up in the Luftkammer, which can be called then generally also means of driving chamber. Then this gas or the fluid pressure changes pulsating in it in each case would have to be subjected. Air can be handled particularly easy however and therefore as particularly suitably for the operation of the bioreactor proved.
Due to the Luftkammer the drive can be arranged very compact for promoting the fluid in the river chamber and be arranged also outside of the Inkubators. Thereby a separation can be caused between the part, which affects the river chamber directly, and which part, that the pressure fluctuations in the Luftkammer, and/or. Means of driving chamber produces. So z can. B. a Respiratorpumpe outside of the Inkubators arranged its, those over a thin hose with the Luftkammer, and/or. Means of driving chamber is connected. Thus the organization of the bioreactor and the drive can be adapted to the respective installation requirements.
According to invention the pulsatile river chamber used for the procedure is a component of a bioreactor. A preferential execution form of the bioreactor becomes following with reference to fig. 2 and 3 described in detail. It understands itself that both the bioreactor and it the comprehensive arrangement could be used not only in the procedure according to invention, but also independently of it.
Show:
Fig. 1a a carrier trained from a polymer, which is subjected afterwards to a settlement with fibroblasts/Myofibroblasten and endothelial cells;
Fig. 1b the carrier/the matrix after settling and one week Inkubation in the pulsatilen river chamber;
Fig. 1K the same flap prosthesis after 2 weeks Inkubation in the river chamber.
Fig. 2 the bioreactor including a pulsatilen river chamber in a cutaway view;
Fig. 3 an arrangement for the operation of the bioreactor from fig. 2.
In fig. a bioreactor 1 is represented 2 in a cutaway view. The bioreactor is around in fig. 2 represented vertical symmetry axis essentially rotationally symmetrically. It concerns with the bioreactor a so-called compact bioreactor, its outside diameters in radial direction approx. 15.5 cm and its height approx. 16.8 cm amounts to. The enterprise can take place also in standard Zellinkubatoren.
The bioreactor 1 orders 2, with two chambers 3 and 4, which are by diaphragm 5 from each other separated over a housing. The lower chamber 3 forms a Luftkammer and the upper chamber 4 a river chamber, which is divided into two parts, whereby a part is formed by a fluid chamber section 6 and the other part by a valve perfusion chamber section 7. The fluid chamber section 6 and the valve perfusion chamber section 7 are connected by a passage 8.
The chambers communicate with the environment over connections 9, 10 and 11, whereby connection 9 into the chamber 3, connection 10 flows into the fluid chamber section 6 of the river chamber 4 and connection 11 into the valve perfusion chamber section 7 of the river chamber 4. The connections 9, 10 and 11 are designed as tube ends managing over the housing in each case, on which hoses or lines are plug-on in well-known way.

The housing is three-part trained, with a lower Gehäuseteil 12, a middle Gehäuseteil 13 and an upper Gehäuseteil 14. Lower Gehäuseteil 12 is essentially dish-shaped with a soil 15 and an essentially circularly running wall 16. Those the wall 16 turned away side of the soil forms at the same time the bearing surface for the bioreactor, with which he z. B. on a table to be touched down can. The connection 9 extends essentially radially from the wall 16 out. The wall 16 exhibits a flange surface 17, in which in axial direction extending tapped holes 18 are let in. In order to facilitate an observing of the procedures inside the chamber 3, is lower Gehäuseteil from transparent material, z. B. Plexiglass (Polymethylmetacrylat, PMMA), manufactured.

Middle Gehäuseteil 13 is likewise essentially rotationally symmetrically had turned away side of the wall 19 around the symmetry axis of the bioreactor and over an essentially cylindrical wall 19, which a cover section 20 follows, as well as a flange 21 on that the cover section 20. The flange 21 is essentially circular with one the flange surface 17 turned flange surface 22, which extends radially. In the flange 21 are besides through-holes 23 intended, aligning one of the tapped holes 18 is assigned to which in each case. The through-holes 23 and tapped holes 18 are evenly distributed at the extent, whereby the preferential execution form has 18 with associated through-holes 23 nine tapped holes. Between the two flange surfaces 17 and 22 is the diaphragm 5. By the diaphragm 5 the two chambers 3 and 4 are separated. The diaphragm consists of high elastic silicone, which is strained in the kind of a drum skin between the flange surfaces 17 and 22. The diaphragm points a thickness from approx. 0.5 mm up, and it can be so trained with the fact that in the installed condition its outside diameter essentially corresponds to the outside diameter of the lower and the middle Gehäuseteils. Then the diaphragm must be provided with openings in the range of the through-holes 23. In order to clamp the diaphragm 5 firmly between the flange surfaces 17 and 22, by the through-holes 23 screws 24 from stainless steel into the tapped holes 18 are screwed in, which extend also by the openings through diaphragm. By the screws 24 the flange surfaces 22 and 17 are pressed against each other, in order to clamp thereby the diaphragm firm and to obtain optimal tightness.

During the enterprise of the bioreactor the diaphragm in axial direction swings. In order to reduce the load of the diaphragm at the transition of the flange surface 17 and 22 to the assigned walls 16 and 19, circulating there in each case phases 25 and 26 are intended.

The fluid chamber section 6 is limited upward thus downward by the diaphragm 5 and by the cover section 20. In order to be able to supervise the reactions in the fluid chamber section 6 visually, middle Gehäuseteil, like also lower Gehäuseteil of transparent material the z consists. B. Plexiglass.

Middle Gehäuseteil 13 follows upper Gehäuseteil 14. This is essentially bell-shaped and by a flange connection with the middle Gehäuseteil connected. For this flange connection middle Gehäuseteil has 13 in its cover section six tapped holes 27, which extend in axial direction and are evenly distributed at the extent. The tapped holes 27 are arranged thereby in a radially running flange surface 28, which exhibits a circulating groove 29 for the admission of a sealing element.

Such a sealing element knows z. B. an O-ring its. The groove 29 is radially inward transferred in relation to the tapped holes 27.
Upper Gehäuseteil 14 has turned flange surface 32 a flange ring 30 with through-holes 31 and one the flange surface 28. Upper Gehäuseteil 14 rises with its flange surface 32 on the flange surface 28 and is by screws 33 from stainless steel, which are put by the through-holes 31 and screwed in in each case into the tapped holes 27, with the middle Gehäuseteil 13 firmly connected. The sealing element is pressed thereby by the flange surface 32 into the groove 29 and seals the valve perfusion chamber section 7 to the environment. The connection 11 is arranged in the point of the upper Gehäuseteils 14. Similar to the two other Gehäuseteile 12 and 13 upper Gehäuseteil consists 14 of transparent material z. B. Plexiglass.
As mentions already initially, the fluid chamber section 6 and the valve perfusion chamber section 7 connected by a passage 8 are. This passage 8 is formed by a through-hole 34 in the cover section 20 of the middle Gehäuseteils 13. Into this through-hole 34, which extends axially, a pipe 35 is pushed in, that itself extended over the cover section 20 outside. On the tubing section of the pipe 35, which extends over the cover section 20 of the middle Gehäuseteils 13 outside, a silicone pipe 36 is attached. This silicone pipe is removable. At the outside diameter of the silicone pipe a circular stage 37 is intended. By the organization of the silicone pipe 36 and the stage 37 it is possible to attach valves to the silicone pipe 36. Also it is possible to put into the silicone pipe 36 valves or filters since the pipe 35 exhibits a smaller inside diameter than the silicone pipe 36 or thus a contact surface for a filter or such a thing the face of the pipe 35 can form.
In the available case the valve is a 2-segeliges or a 3-segeliges flap valve, that not represented more near the carrier described above, and/or. corresponds to the matrix, and as check valve works, in order to make possible a fluid stream only from the fluid chamber section to the valve perfusion chamber section. The Klappenkonstrukt of the flap valve is inserted on a silicone ring. For fastening the flap valve to the silicone pipe the silicone ring is put on the silicone pipe and held by frictional engagement.
At the lower surface of the cover section a circular recess 38 is intended, which runs concentrically to the pipe 35 and flows into those the pipe 35. This recess 38 knows z. B. as admission for filter materials or such a thing serve.
In the further a bioreactor arrangement with the bioreactor 1 described above is described. For the operation of the bioreactor a Respiratorpumpe 39 is intended, which is connected by a silicone hose 40 and the connection 9 with the chamber 3. The Respiratorpumpe produces adjustable pressure impulses, by which the pressure of the chamber can be periodically increased. The Respiratorpumpe is steered two-phases a Respiratorpumpe, which functions as tire pump. With the pump the pumping volume and the pumping frequency can be stopped, whereby the river lies in a range from 50 ml per minute to 5000 ml per minute, and the system pressure between 20 and 240 mmHg to vary can.
A reservoir 41 is connected by silicone hoses 42 and 43 with the connections 10 and 11 in each case. From the silicone hoses 42 and 43 and the reservoir 41 a cycle results, whereby fluid from the valve perfusion chamber section 7 by means

of the connection 10, the silicone hose 42 to the reservoir 41 and from there out by means of the silicone hose 43 can be promoted and the connection 10 to the fluid chamber section 6.
The bioreactor 1 and the reservoir 41 with the silicone hoses 42 and 43 are in a standardized Inkubator 44 with 37 DEG C and 5% CO2.
In the following the effect and function mode of the bioreactor are more near described:
Over the Respiratorpumpe 39 pressure impulses are led into the chamber 3. Due to these pressure impulses the diaphragm expands 5, whereby it itself in the representation in fig. 2 with each pressure pressure upward curves and at it also to an increase of pressure in the fluid chamber section 6 of the river chamber 4 leads. This increase of pressure is passed on into the passage 8 and does not open thereby the flap valve at the silicone pipe 36, represented not more near. In this way the fluid existing in the fluid chamber section 6 is promoted to the valve perfusion chamber section 7. From the valve perfusion chamber section 7 fluid is then promoted by means of the connection 11 and the silicone hose 42 to the reservoir 11. From there out it can be returned by means of the silicone hose 43 to the fluid chamber section 6. With sloping pressure in the chamber 3 the diaphragm 5 is backtransferred again due to its internal voltage into their starting position, into which it essentially radially extends. Via the decrease of pressure a decrease of pressure also in the fluid chamber section 6, which leads again to a reasoning as flap valve of trained valve, takes place.
Thus a cycle can be produced by the pulsating increase of pressure in the chamber, essentially the physiological river conditions in the heart simulated.
Due to the construction of the new bioreactor a high tightness of the fluid chamber section 6 and the valve perfusion chamber section 7 is reached. Due to the protection from infections, possible thereby, long cultivation times can be made possible. By the drive with air over the chamber 3, which is hermetically from the fluid chamber 6 separate by the diaphragm 5, the problems of heat developments can be avoided within the Inkubators 44, there the actual pumping engine (Respiratorpumpe) outside of the Zellinkubators are.
Since the entire bioreactor is transparency, one can see the flap construction permanently and control an opening and a closing of the flaps. Further one can recognize pH changes in the colouring of the nutrient fluid.
The reservoir with the fluid can be attached by sterile connectors to the silicone hoses. Thus a safe fluid change can be made possible.
Due to the simple construction of the bioreactor an exchange of the valves can and/or. the Herzklappen to be accomplished in a simple manner. In addition only the screws 33 must be solved and upper Gehäuseteil 14 be removed. The valve and/or. the Herzklappe can be exchanged then and afterwards can upper Gehäuseteil 14 by the screws 33 again to the middle Gehäuseteil be fastened.
In an execution form of the invention river rates between 50 become ml/min. and 5000 ml/min., prefers 50 ml/min. until 2000 ml/min use. The data refer to the river by the flap prosthesis. As initial river rate river rates from 50 to 100 have themselves ml/min. as suitably proved. These river rates become z. B. with a pulse frequency by 5 to 10 pulses per minute by the Herzklappe skillfully. The

river rate becomes afterwards continuously or intermittent on up to 5000 ml/min. increased. The pulse frequency on up to 180 pulses/min. becomes simultaneous. raised. With the indicated data it concerns the limit values, which are not crossed normally.
In preferential execution forms the river rate becomes up to 2000 ml/min. increased, while the pulse frequency on 70 to 100 prefers, 80 pulses/min. one raises. The load of the stabilizing Herzklappe is adapted thereby to almost physiological conditions. It has itself as favorable, but, the river rate and the pulse frequency not necessarily proved in each case after approximately. to increase 24 to 48 hours. So can for example, on the basis of a river rate from 50 to 100 ml/min. and a pulse rate of 5 to 10 pulses/min. on the day 1 of the stay in the pulsatilen river chamber, on the day 3 an increase on 300 ml/min. with 20 to 25 pulses/min., on the day 5 to 700 ml/min. and 35 to 45 pulses/min., on the day 7 on 1000 ml/min and 50 to 60 pulses/min., on the day 9 on 1300 ml/min. and 70 to 80 pulses/min., on the day 11 on 1500 ml/min. and approx. 100 pulses/min., on the day 13 on 1750 ml/min. and approx. 120 pulses/min. and on the day 15 on 2000 ml/min. and 140 pulses/min. are planned. Depending upon for the order of standing time, size of the flap, size and the age of the patient etc. however a very much slower increase of the river rates as well as the pulse frequency can be meaningful or the increase on higher river rates and pulse frequencies.
In an execution form of the invention the systemic pressures dominant in the pulsatilen river chamber are stopped to 10 to 240 mmHg. Systemic pressures are preferential preferentially from 60 to 140 particularly are systemic pressures from 80 to 120 mmHg.
According to invention the homologous manufactured by means of the procedure and/or. autologe Herzklappe exhibits substantial advantages opposite the conventional mechanical and biological Herzklappen. Thus it consists in its preferential execution form of auto+lied fabrics, D. h. made of fabric of the patient waiting for the heart flap operation, and thereby each foreign body reaction of the flap receiver avoids. The risk of infection with receivers of autologer Herzklappen differs not from the one healthy heart. A Antikoagolationstherapie is not necessary; thus the danger of hemorrhagischer complications is void. The by far most convincing advantage of the Herzklappe according to invention is however the fact that it represents living fabric and therefore after implantation with growth of the heart flap receiver along. That makes the Herzklappe according to invention the flap of the choice particularly with children and juvenile patients, whose heart development still becomes larger. The living Herzklappe grows accordingly also, so that also on changes of the heart no Dysproportionen (z. B. Arise to narrowings) between flap and heart.
The Herzklappe according to invention contains a bindegewebige internal structure, which essentially contains components of a normal extracellular matrix, i.e. Kollagen, a Elastin and a Glycosaminoglykane beside fibroblasts and Myofibroblasten. Contrary to earlier attempts to manufacture by ?tissue engineering? heart flap fabric the flaps according to invention point to one the native flap and/or. the native folding gel appropriate portion of Kollagen (43-55%), Elastin (11-13%) and Glycosaminoglycan up.

It could be shown that the Herzklappen according to invention river rates of more than 2000 ml/min., according to river conditions dominant in an adult human heart, to withstand. Thus a autologe Herzklappe can be made available for the first time according to invention, which is unconditionally for the implantation into childlike like also adults patients suitable.
The following examples describe the invention.
Example 1

Production of carriers

Manufacturing the herzklappenförmigen carrier a non-woven Polyglycolsäurepolymer (fiber diameter becomes: 12-15 mu m, polymer density: 70 mg/ml, Albany internationally Research, Mansfield mA, the USA.) uses. The polymer is cut in the kind that it forms a tube with 19 mm in diameter. In this Conduit 3 triangular sails are inserted. This carrier can for manufacturing 3-segeligen flaps, D. h. Pulmonal, Aorten and Tricuspidalklappen to be used. For Mitralklappen 2 sails are inserted.
Example 2

Production of a dreisegeligen heart flap prosthesis

A dreisegeliger carrier is sterilized and inserted into medium (DMEM, GIBCO BRL running Technologies) for 24 hours, in order to in-soft the polymer surface. Thereupon the klappenförmige carrier per square centimeter becomes surface with 4 millions Fibroblasts every 90 minutes altogether 6 times settles. Further the settled carrier is inkubiert for 2 weeks (5% CO2, 37 DEG C, 95% air humidity). The medium is changed every 4 days under sterile conditions. Subsequently, endothelial cells are applied on the settled klappenförmigen carrier (3-4 millions Endothelial cells per square centimeter surface, 6 settlements every 90 minutes). After further 2 weeks the developed fabric is brought into the river chamber the bioreactor under sterile safeguard clauses and installed here by means of the silicone ring into flow position. Subsequently, the bioreactor is filled with medium and placed into the Zellinkubator. After over the compressed air hose the Konnektion was manufactured to the pump standing outside of the Inkubators, with minimum pulsatilen rivers one begins (50 ml/min). In 2 daily steps the river rate and pulse rate increased on 100 ml/min (pulse 10), 300 ml (pulse 25), 700 ml (pulse 35), 1000 ml (pulse 60) for altogether far 4 days. Subsequently, (after 14 days) the fabric under sterile conditions, formed now, is taken and asserviert to the biochemical, histological and mechanical analysis.